

Voltage dependence of rate functions for Na⁺ channel inactivation within a membrane

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Abstract

The inactivation of a Na⁺ channel occurs when the activation of the charged S4 segment of domain DIV is followed by the binding of an intracellular hydrophobic motif which blocks conduction through the ion pore. The voltage dependence of Na⁺ channel inactivation is, in general, dependent on the rate functions of the S4 sensors of each of the domains DI to DIV. If the activation of a single voltage sensor that regulates the Na⁺ channel conductance is coupled to a two-stage inactivation process, the rate functions for inactivation and recovery from inactivation, as well as the time dependence of the Na⁺ current in terms of the variables $m(t)$ and $h(t)$, may be derived from a solution to the master equation for interdependent activation and inactivation. The rate functions have a voltage dependence that is consistent with the Hodgkin-Huxley empirically determined expressions, and exhibit saturation for both depolarized and hyperpolarized clamp potentials.

INTRODUCTION

The opening and subsequent inactivation of Na⁺ channels and the activation of K⁺ channels generate the action potential in nerve and muscle membranes [1]. The Na⁺ channel transient current during a depolarizing voltage clamp may be described by the expression m^3h where the activation variable m and inactivation variable h satisfy first order rate equations with rate functions dependent on the potential difference across the membrane. Support for the assumption that activation and inactivation are separate processes was provided by the removal of Na⁺ inactivation from the squid axon membrane by the internal perfusion of pronase without affecting activation kinetics [2]. However, there is a delay in the onset of Na⁺ channel inactivation that is dependent on the time-course of channel activation, and Na⁺ channel inactivation partially immobilizes the gating charge associated with activation, and it was assumed that the voltage dependence of inactivation was derived from the Na⁺ activation process [3]. There is also a delay in the recovery from inactivation that is dependent on the time-course of deactivation, and the rate of recovery from inactivation saturates for large hyperpolarizing potentials [4], and therefore, activation and inactivation are interdependent or coupled processes.

The Na⁺ channel protein is comprised of four domains DI to DIV, each containing six alpha-helical segments S1 to S6, and in each domain the voltage sensor, the S4 segment, has positively charged residues located at every third position. The re-entrant loop between S5 and S6 forms the ion-selective filter at the extracellular end of the pore, whereas the intracellular end of the pore is formed by the S6 segments. The inactivation gate is an IMF motif that is positioned on an intracellular loop between DIII and DIV, and interacts with and blocks the flow of ions through the inner mouth of the pore [5]. Based on voltage clamp fluorometry, in response to membrane depolarization, the transverse motion of the charged S4 segments of the Na⁺ channel domains DI to DIII is associated with activation, whereas the slower movement of DIV S4 is correlated with inactivation [6, 7]. This may occur for small depolarizations when the ion channel is usually closed (closed-state inactivation) or for larger depolarizations when the S4 segments of the domains D1 to D3 are activated (open-state inactivation).

In a naturally occurring paramyotonia congenita mutation of the outermost arginine residue of DIV S4 in the human muscle Na⁺ channel, the inactivation rate is decreased with little voltage dependence for moderate depolarizations [8, 9], and therefore, the voltage dependence of inactivation is dependent on charged residues in the S4 segment of the DIV domain. The voltage dependence of the open to inactivated transition was also demonstrated by comparison of gating current measurement in wild-type and ApA toxin modified cardiac Na⁺ channels [10]. By measurement of the OFF gating charge during repolarization in an inactivation modified mutant of the human heart Na⁺ channel, it was estimated that the DIV S4 sensor contributes approximately 30% to the OFF charge, approximately 20% may be attributed to the DIII S4 sensor when the inactivation gate is intact, and the rate-limiting step is the motion of the DIV S4 sensor and not the unbinding of the inactivation gate [11].

In order to account for the effect of double-cysteine mutants of S4 gating charges on the ionic current of the bacterial Na⁺ channel NaChBac, it has been proposed that at least two transitions are required during the activation of each voltage sensor [12]. This conclusion is consistent with an earlier result that cross-linking a DIV S4 segment from the extracellular surface inhibits inactivation during membrane depolarization whereas cross-linking the same segment from the inside inhibits activation of the Na⁺ channel, and therefore, the DIV S4 sensor translocates across the membrane in two stages [13]. A Na⁺ channel model that assumes that the motion of the DIV S4 sensor includes a first stage that is necessary for opening of the channel, and a second stage that is required for inactivation, provides a good description of gating and ionic currents [14]. The measurement of gating currents for charge neutralized segments in each domain of the Na⁺ channel gives additional support to the conclusion that the two stage activation of the DIV S4 sensor is correlated with ion channel inactivation [15].

In this paper, expressions for the voltage dependence of the rate of inactivation and recovery from inactivation are derived by assuming that Na⁺ channel inactivation is a two stage process, where the activation of DIV S4 is correlated with the binding of the inactivation motif to the ion pore. However, Na⁺ channel inactivation is, in general, dependent on the rate functions of the S4 sensors of each of the domains DI to DIV, and from a solution of the master equation for the activation of Na⁺ channel conductance by a single voltage sensor that is coupled to a two-stage inactivation process, it is shown that the voltage dependence of the rate functions for inactivation and recovery from inactivation have a similar form to empirical expressions for Na⁺ channels [1, 4], and in particular, the exponential variation exhibits saturation for both depolarized and hyperpolarized clamp potentials.

INDEPENDENT ACTIVATION AND INACTIVATION OF A Na CHANNEL

Inactivation of a Na⁺ channel may be described as the transverse motion of the charged S4 segment of the domain DIV, with rate functions α_i and β_i , followed by the binding of an intracellular hydrophobic motif which blocks conduction through the ion pore, with rate functions γ_i and δ_i (see Fig. 1). Assuming that the transition of the DIV S4 segment across two potential barriers occurs within an energy landscape, it may be shown from a solution of the Smoluchowski equation [16, 17, 18] that the occupation probabilities of the permissive states h_1 , h_2 and the inactivated state h_3 are determined by

$$\frac{dh_1}{dt} = -\alpha_i h_1(t) + \beta_i h_2(t), \quad (1)$$

$$\frac{dh_2}{dt} = \alpha_i h_1(t) + \delta_i h_3(t) - (\beta_i + \gamma_i) h_2(t), \quad (2)$$

$$\frac{dh_3}{dt} = \gamma_i h_2(t) - \delta_i h_3(t), \quad (3)$$

where each rate is an exponential function of the membrane potential. If the

Na⁺ channel is depolarized to a clamp potential V from a large hyperpolarized holding potential ($h_1(0) = 1$, and $h_2(0) = h_3(0) = 0$), and it is assumed that the first forward and backward transitions are rate-limiting [19, 20] ($\beta_i \gg \delta_i$ and $\gamma_i \gg \alpha_i$), the solution of Eqs. (1) to (3) is [21]

$$h_{3I}(t) = \frac{\alpha_i \gamma_i}{\omega_1 \omega_2} + \frac{\alpha_i \gamma_i}{\omega_1(\omega_1 - \omega_2)} \exp(-\omega_1 t) - \frac{\alpha_i \gamma_i}{\omega_2(\omega_1 - \omega_2)} \exp(-\omega_2 t). \quad (4)$$

where $\omega_1 \approx (\gamma_i \alpha_i + \delta_i(\alpha_i + \beta_i))/(\gamma_i + \beta_i)$ and $\omega_2 \approx \gamma_i + \beta_i \gg \omega_1$.

However, if the DIV S4 sensor is initially in the inactivated state ($h_1(0) = h_2(0) = 0$, and $h_3(0) = 1$), and the membrane is hyperpolarized to a potential V , it may be shown that

$$h_{3D}(t) = \frac{\alpha_i \gamma_i + \delta_i(\alpha_i + \beta_i) \exp(-\omega_1 t)}{\alpha_i \gamma_i + \delta_i(\alpha_i + \beta_i)}. \quad (5)$$

Eqs. (4) and (5) are solutions of the rate equation [1]

$$\frac{dh_3}{dt} = \beta_{h,2} - (\alpha_{h,2} + \beta_{h,2})h_3, \quad (6)$$

where

$$\alpha_{h,2}(V) \approx \frac{\delta_i(\alpha_i + \beta_i)}{\gamma_i + \beta_i}, \quad (7)$$

$$\beta_{h,2}(V) \approx \frac{\alpha_i \gamma_i}{\gamma_i + \beta_i}. \quad (8)$$

Therefore, the probability of the permissive states $h = h_1 + h_2$ satisfies

$$\frac{dh}{dt} = \alpha_{h,2} - (\alpha_{h,2} + \beta_{h,2})h, \quad (9)$$

and Eqs. (7) and (8) provide a good fit to the empirical inactivation rate functions α_h and β_h for the squid axon Na⁺ channel [1] (see Fig. 2).

Based on the measurement of a rising phase of the gating current in a squid axon membrane and the chemical structure of a Na channel, at least two transitions are required for the activation of each voltage sensor [12, 22]. Therefore, for each voltage sensor from domains DI to DIII, assuming no cooperativity between sensors, the occupation probabilities of the closed states m_1 , m_2 and the open state m (see Fig. 4) are determined by

$$\frac{dm_1}{dt} = -\alpha_a m_1(t) + \beta_a m_2(t), \quad (10)$$

$$\frac{dm_2}{dt} = \alpha_a m_1(t) + \delta_a m(t) - (\beta_a + \gamma_a) m_2(t), \quad (11)$$

$$\frac{dm}{dt} = \gamma_a m_2(t) - \delta_a m(t). \quad (12)$$

where the transition rates α_a , β_a , γ_a and δ_a are exponential functions of the membrane voltage V . If we assume that $\beta_a \gg \delta_a$ and $\gamma_a \gg \alpha_a$ [19, 20], from the

solution of Eqs. (10) to (12) during activation ($m_1(0) = 1$), and deactivation ($m(0) = 1$), it may be shown that the open state m may be approximated by

$$m_A(t) \approx \frac{\alpha_a \gamma_a}{\alpha_a \gamma_a + \delta_a(\alpha_a + \beta_a)} [1 - \exp(-\omega_1 t)], \quad (13)$$

$$m_D(t) \approx \frac{\alpha_a \gamma_a + \delta_a(\alpha_a + \beta_a) \exp(-\omega_1 t)}{\alpha_a \gamma_a + \delta_a(\alpha_a + \beta_a)}. \quad (14)$$

where the low frequency $\omega_1 \approx (\gamma_a \alpha_a + \delta_a(\alpha_a + \beta_a))/(\gamma_a + \beta_a)$. Eqs. (13) and (14) satisfy the rate equation [1]

$$\frac{dm}{dt} = \alpha_{m,2} - (\alpha_{m,2} + \beta_{m,2})m, \quad (15)$$

and the rate functions

$$\alpha_{m,2}(V) \approx \frac{\alpha_a}{1 + \beta_a/\gamma_a}, \quad (16)$$

$$\beta_{m,2}(V) \approx \frac{\delta_a(\alpha_a + \beta_a)}{\gamma_a + \beta_a}, \quad (17)$$

provide a good fit to the empirical functions α_m and β_m for the squid axon Na channel [1] (see Fig. 4). However, the cooperativity between the S4 sensors in domains DI to DIII also contributes to the voltage dependence of the Na+ conductance rate functions, and more recent models have adopted exponential functions for both α_m and β_m [4].

COUPLED MODELS OF ACTIVATION AND INACTIVATION OF A Na CHANNEL

The time-dependence of the Na+ current in the squid axon may be expressed as m^3h where the activation variable $m(t)$ and inactivation variable $h(t)$ satisfy the rate equations [1]

$$\frac{dm}{dt} = \alpha_m - (\alpha_m + \beta_m)m, \quad (18)$$

$$\frac{dh}{dt} = \alpha_h - (\alpha_h + \beta_h)h. \quad (19)$$

The Hodgkin-Huxley (HH) description of the Na current is equivalent to an 8-state master equation where three independent voltage sensors may activate, and inactivation may occur from the open state or from each of the closed states [23]. In this section, we assume that the activation of a single voltage sensor regulating the Na channel conductance is coupled to a two-stage inactivation process (see Fig. 5), and therefore, the kinetics may be described by a master equation where the occupation probabilities of the closed states C_1 and A_1 , the open states O and A_2 , and the inactivated (or blocked) states B_1 and B_2 are determined by

$$\frac{dC_1}{dt} = -(\alpha_1 + \alpha_O)C_1(t) + \beta_O O(t) + \beta_1 A_1(t) \quad (20)$$

$$\frac{dO}{dt} = \alpha_O C_1(t) - (\beta_O + \alpha_2)O(t) + \beta_2 A_2(t) \quad (21)$$

$$\frac{dA_1}{dt} = \alpha_1 C_1(t) - (\alpha_A + \beta_1 + \gamma_1)A_1(t) + \delta_1 B_1(t) + \beta_A A_2(t) \quad (22)$$

$$\frac{dA_2}{dt} = \alpha_2 O(t) - (\beta_A + \beta_2 + \gamma_2)A_2(t) + \delta_2 B_2(t) + \alpha_A A_1(t) \quad (23)$$

$$\frac{dB_1}{dt} = \gamma_1 A_1(t) - (\alpha_B + \delta_1)B_1(t) + \beta_B B_2(t) \quad (24)$$

$$\frac{dB_2}{dt} = \gamma_2 A_2(t) + \alpha_B B_1(t) - (\beta_B + \delta_2)B_2(t), \quad (25)$$

and the transition rates are functions of the membrane voltage V .

Assuming that the first forward and backward transitions for inactivation are rate limiting, $\beta_j \gg \delta_j$ and $\gamma_j \gg \alpha_j$, for $j = 1, 2$, A_1 and A_2 satisfy

$$A_1 \approx \frac{\alpha_1 C_1 + \delta_1 B_1}{\beta_1 + \gamma_1}, \quad (26)$$

$$A_2 \approx \frac{\alpha_2 O + \delta_2 B_2}{\beta_2 + \gamma_2}, \quad (27)$$

and therefore, Eqs. (20) to (25) may be reduced to a four state master equation (see Fig. 6)

$$\frac{dC_1}{dt} = -(\rho_1 + \alpha_O)C_1(t) + \beta_O O(t) + \sigma_1 B_1(t) \quad (28)$$

$$\frac{dO}{dt} = \alpha_O C_1(t) - (\beta_O + \rho_2)O(t) + \sigma_2 B_2(t) \quad (29)$$

$$\frac{dB_1}{dt} = \rho_1 C_1(t) - (\alpha_B + \sigma_1)B_1(t) + \beta_B B_2(t) \quad (30)$$

$$\frac{dB_2}{dt} = \rho_2 O(t) + \alpha_B B_1(t) - (\beta_B + \sigma_2)B_2(t), \quad (31)$$

where the derived inactivation rate functions

$$\rho_1 \approx \frac{\alpha_1 \gamma_1}{\beta_1 + \gamma_1}, \rho_2 \approx \frac{\alpha_2 \gamma_2}{\beta_2 + \gamma_2}, \quad (32)$$

$$\sigma_1 \approx \frac{\delta_1(\beta_1 + \alpha_1)}{\beta_1 + \gamma_1}, \sigma_2 \approx \frac{\delta_2(\beta_2 + \alpha_2)}{\beta_2 + \gamma_2}. \quad (33)$$

If Na⁺ activation and inactivation are independent processes ($\alpha_O = \alpha_B$, $\beta_O = \beta_B$, $\rho_1 = \rho_2 = \rho$ and $\sigma_1 = \sigma_2 = \sigma$), and the Na⁺ channel is depolarized to a clamp potential V from a large hyperpolarized holding potential, the open state $O(t) = m(t)h(t)$ where the activation and inactivation variables

$$m(t) = \frac{\alpha_O}{\alpha_O + \beta_O} (1 - \exp[-(\alpha_O + \beta_O)t]), \quad (34)$$

$$h(t) = \frac{\sigma + \rho \exp(-(\rho + \sigma)t)}{\sigma + \rho}, \quad (35)$$

and satisfy the rate equations (18) and (19) when $\alpha_O = \alpha_m$, $\beta_O = \beta_m$, $\rho = \beta_h$ and $\sigma = \alpha_h$ (see Fig. 7). Similarly, if the Na+ channel is hyperpolarized to a clamp potential V from a large depolarized holding potential, $C_1(t) = (1 - m(t))h(t)$ (see Fig. 8) where

$$m(t) = \frac{\alpha_O + \beta_O \exp(-(\alpha_O + \beta_O)t)}{\alpha_O + \beta_O}, \quad (36)$$

$$h(t) = \frac{\sigma}{\rho + \sigma}(1 - \exp[-(\rho + \sigma)t]). \quad (37)$$

In the HH model, the inactivation rate functions are assumed to be independent of the Na+ conductance activation functions but, by contrast, the rate of recovery for inactivation in hippocampal neurons is also dependent on the Na+ channel deactivation functions [4].

More generally, the Na+ current during deactivation of the channel is very small [3], and therefore, the deinactivation rate $\sigma_2 \approx 0$. For a depolarizing clamp potential V from a large hyperpolarized holding potential, the solution of Eqs. (28) to (31) when $\rho_1 = \rho_2 = \rho$ is

$$C_1(t) = C_{1s} + \sum_{j=1}^3 k_{j+1}(\omega_j - \beta_O - \rho_2) \exp(-\omega_j t) \quad (38)$$

$$O(t) = O_s - \sum_{j=1}^3 k_{j+1} \alpha_O \exp(-\omega_j t) \quad (39)$$

$$B_1(t) = B_{1s} + \sum_{j=1}^3 k_{j+1} X_1(\omega_j) \exp(-\omega_j t) \quad (40)$$

$$B_2(t) = B_{2s} + \sum_{j=1}^3 k_{j+1} X_2(\omega_j) \exp(-\omega_j t), \quad (41)$$

where $C_{1s} = k_1 \sigma_1 \beta_B (\beta_O + \rho)$, $O_s = k_1 \sigma_1 \beta_B \alpha_O$, $B_{1s} = k_1 \rho \beta_B (\alpha_O + \beta_O + \rho)$, $B_{2s} = k_1 \rho \alpha_B (\alpha_O + \beta_O + \rho) + \rho \alpha_O \sigma_1$, $k_1^{-1} = [(\alpha_O + \beta_O + \rho)(\sigma_1 \beta_B + \rho(\alpha_B + \beta_B)) + \rho \sigma_1 \alpha_O]$,

$$k_2 = \frac{1 - k_1 \sigma_1 \beta_B \omega_2 + k_4 (\omega_2 - \omega_3)}{\omega_1 - \omega_2} \quad (42)$$

$$k_3 = \frac{1 - k_1 \sigma_1 \beta_B \omega_1 + k_4 (\omega_1 - \omega_3)}{\omega_2 - \omega_1} \quad (43)$$

$$k_4 = \frac{-B_{1s}(\omega_2 - \omega_1) + r_2 X_1(\omega_1) - r_1 X_1(\omega_2)}{(\omega_2 - \omega_1) X_1(\omega_3) + (\omega_3 - \omega_2) X_1(\omega_1) - (\omega_3 - \omega_1) X_1(\omega_2)}, \quad (44)$$

$$X_1(\omega) = \frac{-\rho \alpha_O \beta_B - \rho(\omega - \beta_B)(\omega - \beta_O - \rho)}{\omega^2 - \omega(\alpha_B + \beta_B + \sigma_1) + \sigma_1 \beta_B}, \quad (45)$$

$$X_2(\omega) = \frac{-\rho \alpha_B (\alpha_O + \beta_O + \rho) - \rho \alpha_O \sigma_1 + \rho \omega (\alpha_O + \alpha_B)}{\omega^2 - \omega(\alpha_B + \beta_B + \sigma_1) + \sigma_1 \beta_B}. \quad (46)$$

For depolarization clamp potentials, $\omega_2 \approx \alpha_O + \beta_O + \rho$, $\omega_3 \approx \alpha_B + \beta_B + \sigma_1$, and

$$\omega_1 \approx \alpha_h + \beta_h, \quad (47)$$

where the inactivation rate

$$\beta_h = \frac{\rho}{1 + \sigma_1/(\alpha_B + \beta_B)} + \frac{\rho \sigma_1 \alpha_O}{(\alpha_O + \beta_O + \rho)(\alpha_B + \beta_B + \sigma_1)}, \quad (48)$$

and the rate of recovery from inactivation

$$\alpha_h = \frac{\beta_B \sigma_1}{\alpha_B + \beta_B + \sigma_1}. \quad (49)$$

The second term in Eq.(48) only makes a contribution to β_h for small clamp potentials. If $\alpha_1 = \alpha_2$ and δ_1 are independent of V , ω_1 saturates for both large positive and negative clamp potentials (see Fig. 9). From Eq. (39), as $k_4 \approx 0$ for a depolarizing potential, we may write

$$O(t) \approx \frac{\alpha_O}{\alpha_O + \beta_O} \left(\frac{\alpha_h(1 - \exp[-(\alpha_O + \beta_O + \rho)t])}{\alpha_h + \beta_h} + \right. \quad (50)$$

$$\left. \frac{\beta_h \exp[-(\alpha_h + \beta_h)t](1 - \exp[-(\alpha_O + \beta_O)t])}{\alpha_h + \beta_h} \right), \quad (51)$$

and therefore, $O(t) \approx m(t)h(t)$ (see Fig. 10) where $m(t)$ is defined in Eq. (34) and

$$h(t) = \frac{\alpha_h + \beta_h \exp(-(\alpha_h + \beta_h)t)}{\alpha_h + \beta_h}. \quad (52)$$

That is, the HH description of the Na⁺ current in terms of the variables $m(t)$ and $h(t)$ is an approximation to an expression that may be derived from a solution to a coupled model of Na⁺ activation and two stage inactivation for which the the deinactivation rate $\sigma_2 \approx 0$.

For a moderate hyperpolarizing clamp potential from a depolarized holding potential, the inactivation rates $\rho_1, \rho_2 \approx 0$, and the solution of Eqs. (28) to (31) is (see Fig. 11)

$$C_1(t) = \frac{\beta_O}{\alpha_O + \beta_O} + \frac{Y_1(\omega_1)}{\omega_1 - \omega_2} \exp(-\omega_1 t) - \frac{Y_1(\omega_2)}{\omega_1 - \omega_2} \exp(-\omega_2 t) + \frac{\alpha_O \omega_1 \omega_2 + \omega_3 \sigma_2 (\beta_O - \alpha_B - \sigma_1)}{\omega_3 (\omega_1 - \omega_3)(\omega_2 - \omega_3)} \exp(-\omega_3 t) \quad (53)$$

$$O(t) = \frac{\alpha_O}{\alpha_O + \beta_O} + \frac{Y_2(\omega_1)}{\omega_1 - \omega_2} \exp(-\omega_1 t) - \frac{Y_2(\omega_2)}{\omega_1 - \omega_2} \exp(-\omega_2 t) - \frac{\alpha_O \omega_1 \omega_2 + \omega_3 \sigma_2 (\beta_O - \alpha_B - \sigma_1)}{\omega_3 (\omega_1 - \omega_3)(\omega_2 - \omega_3)} \exp(-\omega_3 t), \quad (54)$$

$$B_1(t) = -\frac{\beta_B}{\omega_1 - \omega_2} \exp(-\omega_1 t) + \frac{\beta_B}{\omega_1 - \omega_2} \exp(-\omega_2 t), \quad (55)$$

$$B_2(t) = \frac{\omega_1 - \alpha_B - \sigma_1}{\omega_1 - \omega_2} \exp(-\omega_1 t) - \frac{\omega_2 - \alpha_B - \sigma_1}{\omega_1 - \omega_2} \exp(-\omega_2 t), \quad (56)$$

where $\omega_3 = \alpha_O + \beta_O$, and ω_1, ω_2 are solutions of the characteristic equation

$$\omega^2 - \omega(\alpha_B + \beta_B + \sigma_1 + \sigma_2) + \sigma_1 \beta_B + \sigma_2(\alpha_B + \sigma_1) = 0, \quad (57)$$

$$Y_1(\omega) = \frac{-\beta_O \omega_1 \omega_2 + \omega(\sigma_1 \beta_B + \sigma_2 \beta_O)}{\omega(\omega - \alpha_O - \beta_O)}, \quad (58)$$

$$Y_2(\omega) = \frac{\omega_1 \omega_2 (\sigma_2 - \alpha_O) - \omega \sigma_2 (\beta_B + \sigma_2 - \alpha_O)}{\omega(\omega - \alpha_O - \beta_O)}. \quad (59)$$

If $\rho_1 = \rho_2$, from the application of microscopic reversibility to the four state system (see Fig. 6), $\beta_B = \beta_O \alpha_B \sigma_2 / \alpha_O \sigma_1 \ll \beta_O$ for $\sigma_1 \gg \sigma_2 \approx 0$. From Eq. (57), assuming that $\alpha_B \ll \beta_B \ll \sigma_1$ for a small hyperpolarizing potential, the lowest frequency $\omega_1 \approx \beta_B$, whereas for $\beta_B \gg \sigma_1$, $\omega_1 \approx \sigma_1$ (see Fig. 9). The conclusion that, for small hyperpolarizing potentials, the recovery rate for inactivation $\alpha_h \approx \beta_B \propto \beta_m$ is supported by the HH data where $\alpha_h(V)$ and $\beta_m(V)$ have a similar voltage dependence and $\beta_m(V) \approx 57\alpha_h(V)$ [1]. However, if α_B is an exponential function of V such that $\alpha_B + \beta_B \gg \sigma_1$ [4] (see Fig. 12), from Eq. (57), $\omega_2 \approx \alpha_B + \beta_B + \sigma_1$, and

$$\omega_1 \approx \frac{\sigma_1 \beta_B}{\alpha_B + \beta_B + \sigma_1}. \quad (60)$$

Therefore, the voltage dependence of the rate of recovery from inactivation is determined by the deinactivation rate σ_1 and the Na⁺ conductance deactivation functions [4]. For small hyperpolarizing potentials ($\beta_B \ll \alpha_B$), $\omega_1 \approx \sigma_1 \beta_B / (\alpha_B + \sigma_1)$, and may be approximated by an exponential function of V [1] but saturates at a more negative potential when $\beta_B \gg \sigma_1 \gg \alpha_B$ (see Fig. 12).

From Eq. (53), we may write

$$C_1(t) \approx \frac{\beta_O}{\alpha_O + \beta_O} \left(1 - \exp(-\omega_1 t) \left[1 + \frac{\omega_1 (1 - \exp[-(\omega_2 - \omega_1)t])}{\omega_2 - \omega_1} \right] \right). \quad (61)$$

and therefore, $dC_1/dt(0) = 0$ and there is a delay in the recovery from inactivation [4, 15]. However, for large negative potentials, $\omega_2 \approx \beta_B \gg \omega_1 \approx \sigma_1$, and Eq. (61) reduces to the HH expression

$$C_1(t) \approx [1 - m_s]h(t), \quad (62)$$

where $m_s = \alpha_O / (\alpha_O + \beta_O)$ and $h(t) = 1 - \exp(-\omega_1 t)$.

CONCLUSION

Hodgkin and Huxley described the voltage dependence of the Na⁺ channel inactivation rate and the rate of recovery by exponential functions which for the inactivation rate saturates for a moderate depolarizing potential [1]. Based on the absence of a gating current that corresponds to the time course of the inactivated Na⁺ current, it was assumed that the transition rates governing inactivation were voltage-independent, and that the macroscopic voltage dependence of inactivation derived from the Na⁺ channel activation process [3]. However, the voltage dependence of the inactivation rate is dependent on the charge on the S4 segment residues of the DIV domain [8, 9, 10], and generally, only has a minor contribution from the Na⁺ conductance activation rate functions. The voltage dependence of the rate of recovery from inactivation saturates for a large hyperpolarizing potential, and has been attributed to the Na⁺ channel deactivation rate functions [4].

In this paper, assuming that Na⁺ channel inactivation is a two stage process, where the activation of DIV S4 is correlated with the binding of the inactivation

motif to the ion pore, we show that during a voltage clamp of the Na⁺ channel, the solution of the master equation for the inactivation process may be approximated by the solution of a rate equation. The backward transition rate is, in general, an exponential function of the membrane potential V , and the forward rate may be expressed as an exponential function for small depolarizations but approaches a saturated value for a larger clamp potential, reflecting the voltage independence of the rate limiting step.

If the processes of Na activation and inactivation are independent, and the activation of a single voltage sensor that regulates the Na channel conductance is coupled to a two-stage inactivation process, the open state probability $O(t)$ during a depolarizing clamp potential, derived from an analytical solution of a four state master equation, may be expressed as $m(t)h(t)$ where $m(t)$ and $h(t)$ satisfy rate equations for activation and inactivation, and the voltage dependence of the rate of inactivation provides a good approximation to the HH function β_h [1]. However, the voltage dependence of the rate of recovery from inactivation is dependent on the rate functions for the DIV sensor and not the Na⁺ channel conductance deactivation functions, as observed experimentally in hippocampal neurons [4].

Based on the measurement of a very small ion channel current during deactivation [4], the Na⁺ channel deactivates before recovery from inactivation, and hence the deinactivation rate σ_2 to the open state is smaller than the corresponding rate σ_1 from a deactivated state. A more general expression for the voltage dependence of the rate of recovery from inactivation may be determined that is approximated by an exponential function for physiological potentials [1], but for more negative hyperpolarizing potentials, it approaches a limiting value equal to the deinactivation rate σ_1 . The inactivation rate is, in general, also dependent on the Na⁺ conductance activation rate functions, as well as the rate functions for the DIV S4 sensor, but for a moderate depolarizing potential it reduces to a two-stage expression when the DIV S4 activation rate $\rho_1 = \rho_2$.

We conclude that general expressions for the voltage dependence of rate functions for inactivation and recovery from inactivation may be determined from a master equation for interdependent Na⁺ channel activation and inactivation, and are consistent with the empirical data from the squid axon [1], hippocampal neurons [4] and Nav1.4 ion channels [15]. When Na⁺ conductance activation is regulated by a single voltage sensor, the HH description of the Na⁺ current in terms of the activation and inactivation variables $m(t)$ and $h(t)$, is an approximation to an expression derived from the solution to a coupled model of Na⁺ activation and two-stage inactivation where the deinactivation rate to the open state $\sigma_2 \approx 0$.

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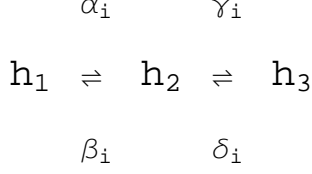


Figure 1: Inactivation model of a Na⁺ ion channel, where the occupation probabilities of the permissive states $h_1(t)$, $h_2(t)$, and the inactivated state $h_3(t)$ satisfy a master equation, and the rate functions α_i , β_i , γ_i and δ_i , are voltage-dependent rate functions between states.

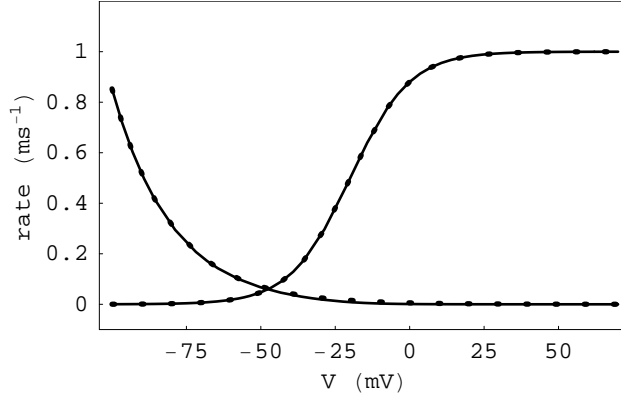


Figure 2: The derived rate functions $\alpha_{h,2}(V)$ and $\beta_{h,2}(V)$ (solid line) in Eqs. (7) and (8) provide a good approximation to the HH rate functions (ms^{-1}) $\alpha_h = 0.07 \exp(-(V+50)/20)$ and $\beta_h = 1/(1 + \exp(-(20+V)/10))$ (dotted line) when the inactivation rate functions are $\alpha_i(V) = 1 \ll \gamma_i(V) = \exp(3)$, and $\beta_i(V) = \exp(-2.5(V-10)/25) \gg \delta_i(V) = 0.07 \exp(-(V+50)/20)$.

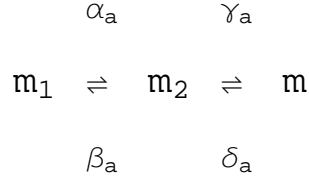


Figure 3: Activation model of a Na⁺ channel, where the occupation probabilities of the closed states m_1 , m_2 , and the open state m satisfy a master equation, and α_a , β_a , γ_a and δ_a are voltage-dependent rate functions between states.

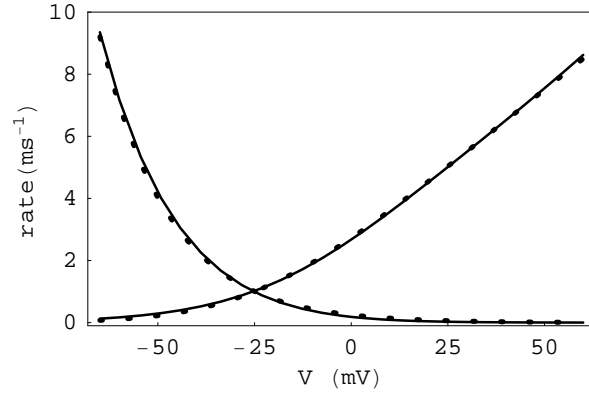


Figure 4: The derived rate functions $\alpha_{m,2}(V)$ and $\beta_{m,2}(V)$ (solid line) in Eqs. (16) and (17) provide a good approximation to the HH rate functions (ms^{-1}) $\alpha_m = 0.1(V+25)/(1+\exp[-0.1(V+25)])$ and $\beta_m = 4 \exp[-(V+50)/18]$ (dotted line) when the activation rate functions are $\alpha_a(V) = 2.7 \exp(0.27(V+50)/25) \ll \gamma_a(V) = 30 \exp(0.25(V+50)/25)$, and $\beta_a(V) = 251 \exp(-0.95(V+50)/25) \gg \delta_a(V) = 4.65 \exp(-0.9(V+50)/18)$.

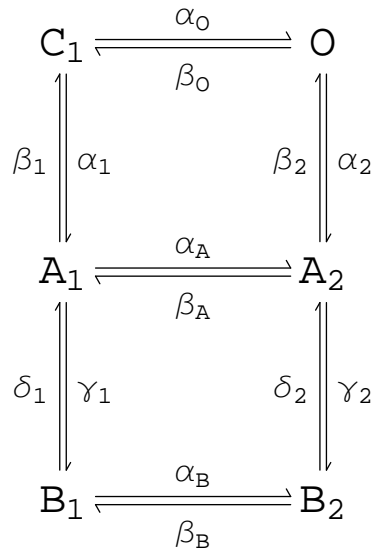


Figure 5: Six state system that describes Na⁺ conductance activation between states C_1 and O , A_1 and A_2 , and B_1 and B_2 , is coupled to a two-stage Na⁺ inactivation process between states C_1 and B_1 , and O and B_2 .

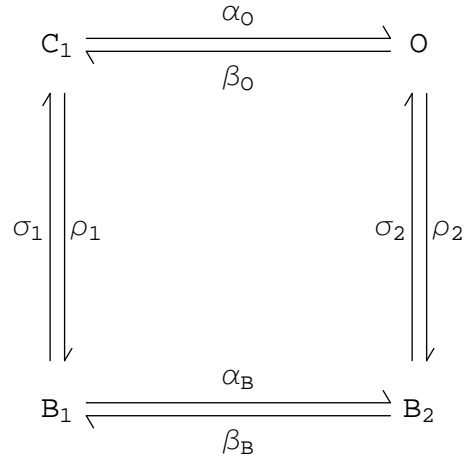


Figure 6: The six state system of Fig. 5 may be approximated by a four state system when $\beta_j \gg \delta_j$ and $\gamma_j \gg \alpha_j$, for $j = 1, 2$ where ρ_j and σ_j are derived rate functions for a two-stage Na⁺ inactivation process, defined in Eqs. (33) and (33).

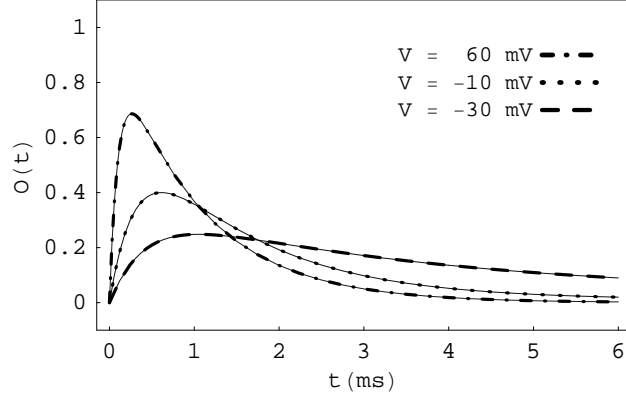


Figure 7: For a four state system where activation and inactivation are independent, the open state probability $O(t)$ (solid line) during a depolarizing clamp potential, is equal to $m(t)h(t)$ (dashed, dotted or dot-dashed line) where $m(t)$ and $h(t)$ are solutions of rate equations for activation and inactivation, and $\alpha_O = \alpha_m = 0.1(V + 25)/(1 - \exp[-(V + 25)/10])$, $\beta_O = \beta_m = 4 \exp[-(V + 50)/18]$, $\rho = \beta_h = 1/(1 + \exp[-(20 + V)/10])$ and $\sigma = \alpha_h = 0.07 \exp[-(V + 50)/20]$ (ms^{-1}) [1].

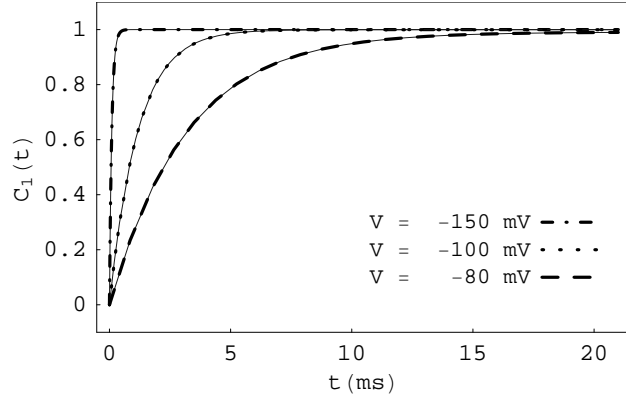


Figure 8: For a four state system where activation and inactivation are independent, the closed state probability $C_1(t)$ (solid line) during a hyperpolarizing clamp potential, is equal to $[1 - m(t)]h(t)$ (dashed, dotted or dot-dashed line) where $m(t)$ and $h(t)$ are solutions of rate equations for activation and inactivation, and $\alpha_O = \alpha_m = 0.1(V + 25)/(1 - \exp[-(V + 25)/10])$, $\beta_O = \beta_m = 4 \exp[-(V + 50)/18]$, $\rho = \beta_h = 1/(1 + \exp[-(20 + V)/10])$ and $\sigma = \alpha_h = 0.07 \exp[-(V + 50)/20]$ (ms^{-1}).

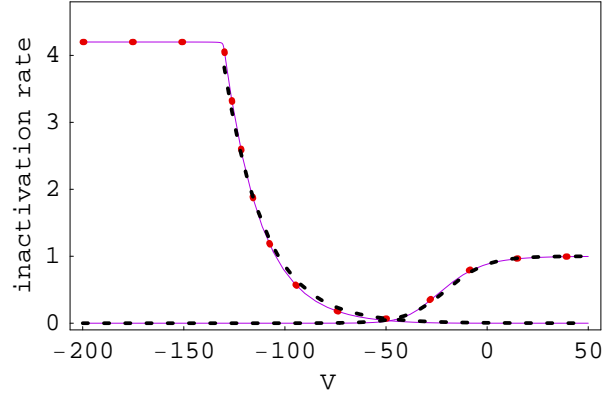


Figure 9: Voltage dependence of the HH Na+ channel inactivation rate functions $\beta_h = 1/(1 + \exp(-(20 + V)/10))$ and $\alpha_h = 0.07 \exp(-(V + 50)/20)$ (dashed line) may be approximated by analytical expressions in Eqs. (48) and (49) (solid line) derived from a master equation for a four state system where activation and two stage inactivation are interdependent, and by the voltage dependence of the lowest frequency of the system determined numerically (dotted line) where the rate functions are $\alpha_1(V) = \alpha_2(V) = 1$, $\gamma_1(V) = \gamma_2(V) = \exp(3)$, $\beta_1(V) = \beta_2(V) = \exp(-2(V - 10)/25)$, $\delta_1(V) = 4.2$, $\delta_2(V) = 0$, $\alpha_O = 0.1(V + 25)/(1 - \exp[-(V + 25)/10]) = \alpha_B/3$ and $\beta_O = 4 \exp[-(V + 50)/18] = 83\beta_B$ (ms^{-1}).

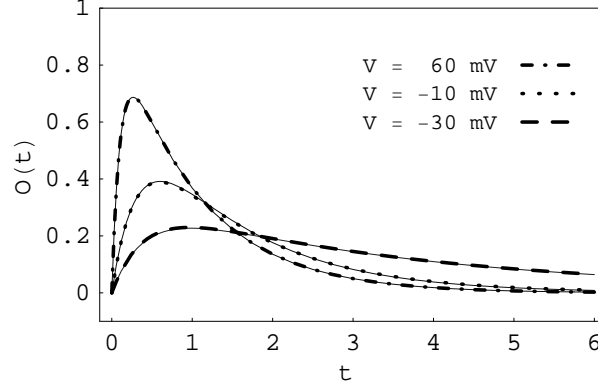


Figure 10: For a four state system where activation and inactivation are interdependent, during a depolarizing clamp potential, the open state probability $O(t)$ (solid line) may be approximated by $m(t)h(t)$ (dashed, dotted or dot-dashed line) where $m(t)$ and $h(t)$ are solutions of rate equations for activation and inactivation, and $\alpha_1(V) = \alpha_2(V) = 1$, $\gamma_1(V) = \gamma_2(V) = \exp(3)$, $\beta_1(V) = \beta_2(V) = \exp(-2(V - 10)/25)$, $\delta_1(V) = 4.2$, $\delta_2(V) = 0$, $\alpha_O = 0.1(V + 25)/(1 - \exp[-(V + 25)/10]) = \alpha_B/3$ and $\beta_O = 4 \exp[-(V + 50)/18] = 83\beta_B$ (ms^{-1}).

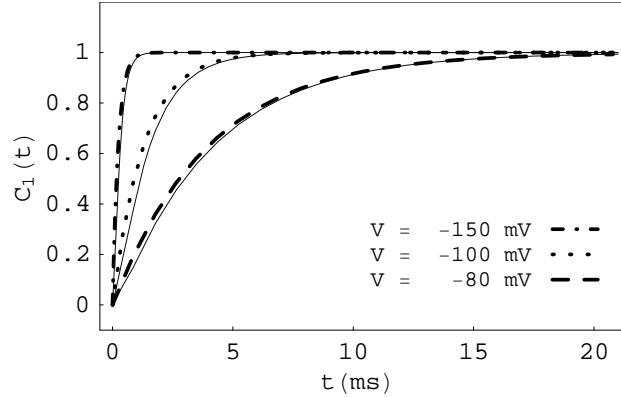


Figure 11: For a hyperpolarizing clamp potential of a four state system where activation and inactivation are interdependent, the closed state variable $C_1(t)$ (solid line) may be approximated by $[1 - m_s]h(t)$ (dashed, dot or dot-dashed) where $m_s = \alpha_O/(\alpha_O + \beta_O)$ and $h(t)$ is a solution of a rate equation for inactivation, and rate functions are $\alpha_1(V) = \alpha_2(V) = 1$, $\gamma_1(V) = \gamma_2(V) = \exp(3)$, $\beta_1(V) = \beta_2(V) = \exp(-2(V - 10)/25)$, $\delta_1(V) = 4.2$, $\delta_2(V) = 0$, $\alpha_O = 0.1(V + 25)/(1 - \exp[-(V + 25)/10]) = \alpha_B/3$ and $\beta_O = 4 \exp[-(V + 50)/18] = 83\beta_B$ (ms^{-1}).

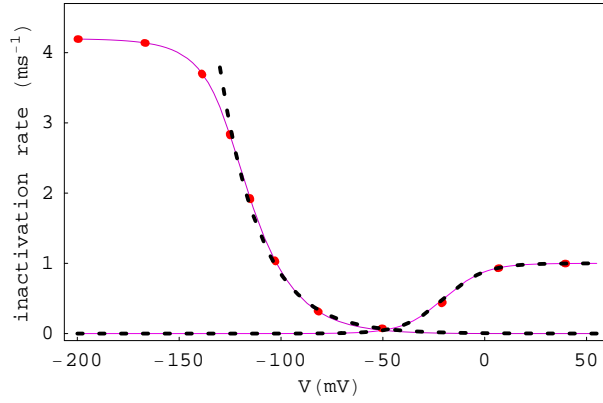


Figure 12: Voltage dependence of the HH Na⁺ channel inactivation rate functions $\beta_h = 1/(1 + \exp(-(20 + V)/10))$ and $\alpha_h = 0.07 \exp(-(V + 50)/20)$ (dashed line) may be approximated by analytical expressions (solid line) derived from a master equation for a four state system where activation and two stage inactivation are interdependent, and by the voltage dependence of the lowest frequency of the system determined numerically (dotted line) where the rate functions are $\alpha_1(V) = \alpha_2(V) = 1$, $\gamma_1(V) = \gamma_2(V) = \exp(3)$, $\beta_1(V) = \beta_2(V) = \exp(-2.5(V - 10)/25)$, $\delta_1(V) = 4.2$, $\delta_2(V) = 0$, $\alpha_O = \alpha_B = 5.4 \exp(-0.3V/25)$ and $\beta_O = 0.5 \exp[-V/18] = 98\beta_B$ (ms^{-1}).